# Functional Protein detection for DNA Mismatch Repair: A Novel Nano-biosensor for Cancer Diagnostics

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## Abstract

Cancer currently stands as the second-leading cause of death worldwide. Studies reveal colorectal cancer (CRC) to be the 4th leading cause of mortality due to cancer. It is estimated that about 30% of CRC cases are hereditary, of which 5% are attributed by known syndromes, particularly Lynch Syndrome. Lynch Syndrome (LS) is caused by loss or malfunction of proteins responsible for DNA mismatch repair proteins (MMR), mostly MLH1 and MSH2, causing increased risks of developing CRC. Despite the small percentage accounted with the disease, the severity of the illness still remains immense since 80% of these patients eventually develop CRC and an overwhelming 40-60 % of female patients develop endometrial cancer, the major of cancer in women in developing nations. Current diagnostic procedures for LS involve testing tumor tissue for microsatellite instability and the presence/absence of MMR proteins by immunohistochemistry (IHC), followed by germine testing for mutations in MMR genes, if warranted. While genetic testing is becoming more cost-effective and accessible, a major problem with this approach is that the functional and pathological consequences of a majority of mutations and small insertions/deletions in MMR genes are unknown, rendering the tests results inconclusive in many cases. Therefore the need for an accurate means of early diagnosis is clearly imminent to prevent the growing threat. This pilot study aims to fabricate a DNA-graphene-polypyrole (DGP) based biosensor to diagnose deficiency of functional MMR proteins present in patients at a scale of less than ng/ ml. Biotinylated DNA probes are immobilized on an avidin film coated over a polypyrole-graphene layer, which in turn is finely deposited over a gold-plated circuit using electrochemical polymerization technique. A detailed morphological characterization has been performed using both scanning electron microscope (SEM) and atomic force microscope (AFM) topography of the DGP biosensor. Quantitative analysis is performed by measuring electrochemical impedance spectroscopy (EIS) where the change in impedance of the samples is recorded by varying frequency between 1MHz- 100 MHz. Though still a work in progress, we assume that the attachment of the MMR proteins onto the mismatched sequences should alter the impedance of the fabricated DGP biosensor. A final analysis is being conducted to confirm our assumptions.

## 1. Method

The complete process for fabrication of the DGP biosensor is illustrated in the flowchart below.

1. Graphene film formation via electropolymerization of polypyrole

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2. Deposition of Avidin

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3. Immobilization biotinylated DNA probes

**Figure 1:** *Process clowchart*

### 1.1 Graphene-Ppy film formation

For this step, we used cyclic voltammetry to co-electropolymerize pyrole and graphene and deposit it on the gold-coated circuit.[1]
CV parameters:

- Vup = 900mV
- Vdown = 800mV
- Scan rate 20 mV/s
- Number of cycles= 100

### 1.2 Deposition of Avidin

Avidin molecules were dissolved in a buffer solution of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid solution (HEPES, 10 mM, pH 7.0). The graphene-Ppy chip was then soaked in the resultant solution for 30 minutes.[2]

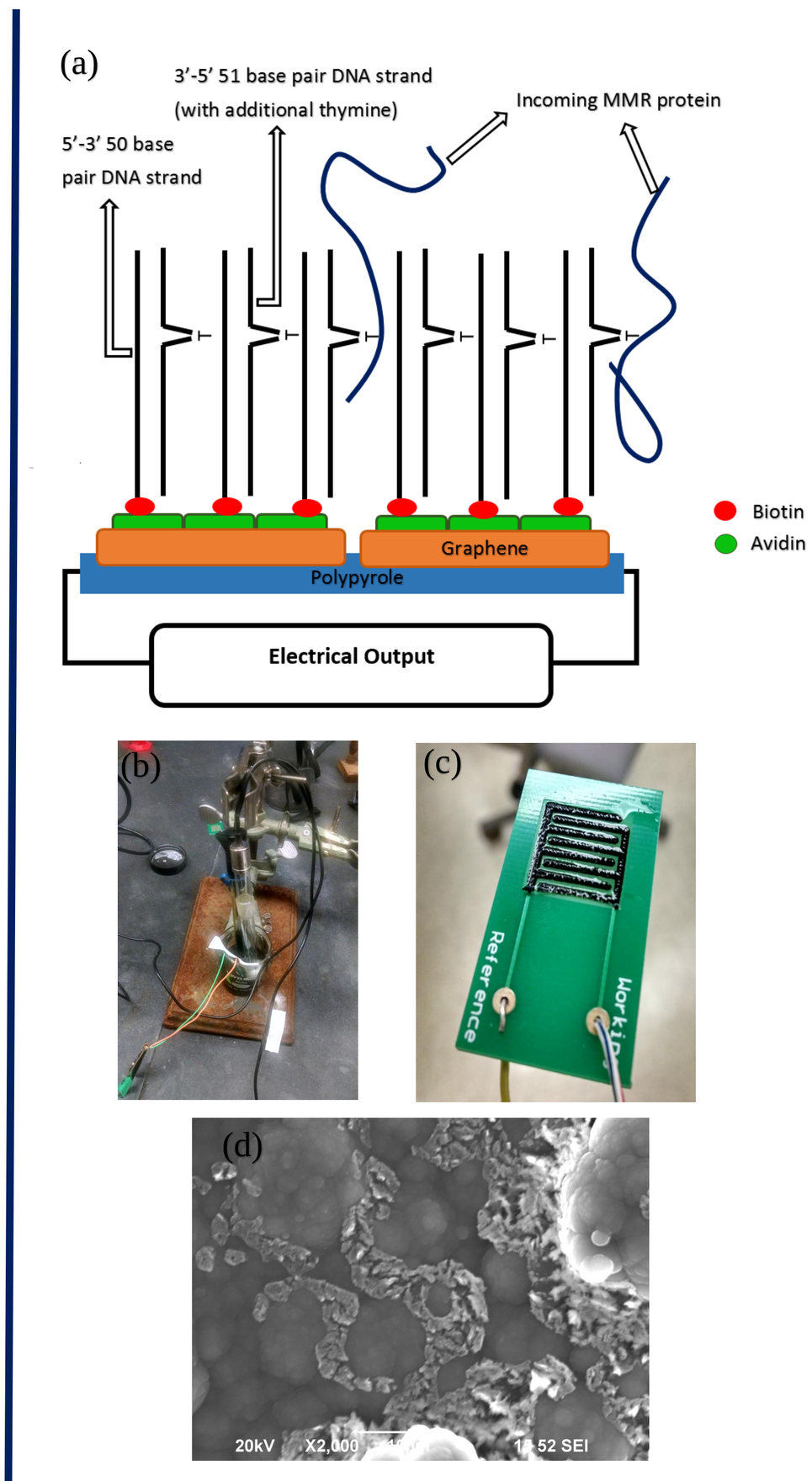


**Figure 2**: *(a) Schematic of final assembled device; (b) Electropolymerization of graphene and pyrole on chip; (c) Finely deposited graphene-polypyrole chip; (d) AFM topography image of graphene-polypyrole surface; (e)SEM of graphene-Ppy surface.*

## 2. Result & Progress

AFM topography reveals successful deposition of Avidin onto the graphene-Ppy surface. EIS studies show different conductance traits between the graphene-Ppy only and graphene-Ppy-Avidin chips.

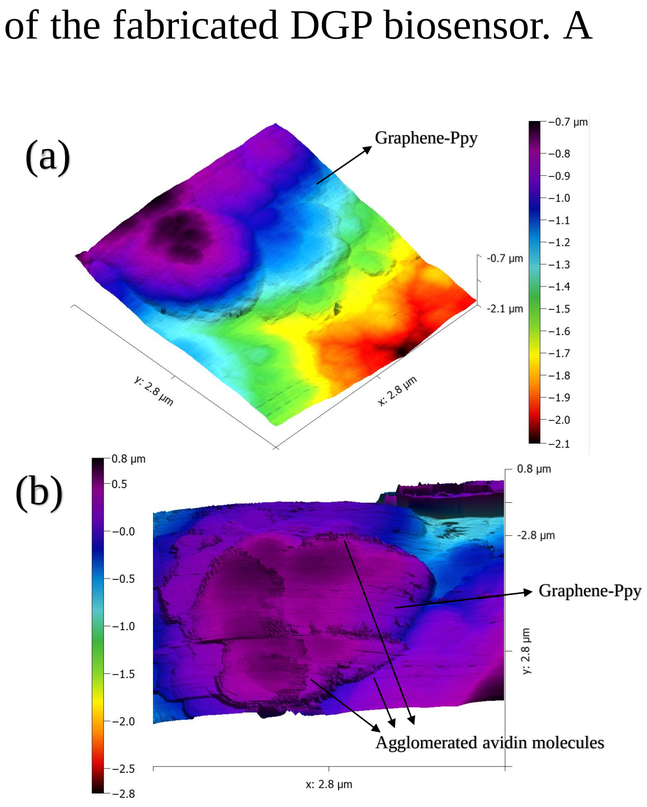


**Figure 3**: *AFM topography of (a) Graphene-Ppy only chip, (b) Graphene-Ppy-Avidin chip*

## 3. Conclusion

At this stage of development, we have been successful in depositing avidin onto the graphene-polypyrole surface, which also show different conductivity compared to graphene-Ppy only chips. Immobilization of the biotinylated DNA probes will be conducted after current tests to optimize the deposition of avidin have been completed.

## 4. Acknowledgements

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- AFM: Yuan You, Dr. Saion Sinha, University of New Haven

## 5. References

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